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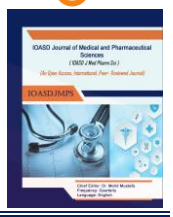

Assessment of Detection of *Helicobacter pylori* by Using the Stool Antigen and Blood Antibody Tests among Patients with Upper Gastrointestinal Disorders in Western Libya

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ABSTRACT

Background: The stomach mucosa is home to the Gram-negative, microaerophilic bacterium *Helicobacter pylori*. Numerous illnesses of the digestive tract, such as gastritis, peptic ulcer disease, gastric cancer, and mucosa associated lymphoid tissue lymphoma, are frequently linked to colonization with this pathogen. The most common methods used for diagnosis of H. pylori infection are serum IgG and IgA antibodies and stool antigen assays. ***Objectives:*** The aim of the study is to compare between stool antigen and blood antibody test (IgG and IgM) method for detection of *Helicobacter pylori* which associated with several upper gastrointestinal disorder among patients in Western Libya. ***Methodology:*** This study included 50 serum and stool specimens were collected from patients at the same day which containing 28 females and 22 males, their aged ranged from 10 to 70 years The data were obtained by questionnaire. The stool samples were analysed for *H. pylori* antigen using *H. pylori* antigen rapid test cassette kit, while the serum were analysed for IgG and IgM antibodies using Premier enzyme immunoassay. ***Results:*** Results of this study showed that prevalence of the infection increased with age greater than 21 years. In the present study stool specimens and serum samples were subjected to examination for detection of Antigen and Antibody respectively. Antibody was detected in 32 out of the 50 samples tested (64%) for IgG and 24 out of 50 samples tested (48%) for IgM. whereas Stool Antigen was positive in 23 (46%) out of 50 samples tested. The current study reveals that IgG showed a slightly greater number of positive cases than IgM and stool antigen test. ***Conclusion:*** Blood antibodies test method showed greater number of positive cases (64%) than the stool antigen tests method (46%) which may be due to past infection. Stool antigen test, which detects present but not previous infection of H. pylori, would be more accurate than serology test.

Keywords: *Helicobacter pylori*, Upper gastrointestinal disorder, Stool antigen test, Blood antibody test, IgG, IgM, Western Libya.

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INTRODUCTION

The stomach mucosa is home to the Gram-negative, microaerophilic bacterium *Helicobacter pylori* (Venerito *et al.,* 2016; Robinson *et al.,* 2017). Numerous illnesses of the digestive tract, such as gastritis, peptic ulcer disease (10–15%), gastric cancer (1–3%), and mucosa associated lymphoid tissue lymphoma (< 0.1%), are frequently linked to colonization with this pathogen (Dunne *et al,* 2014).

Numerous variables influence the kind and severity of disorders, including the host's immune system's condition, the pathogenicity of *H. pylori* strains, and the existence of environmental factors (such as food, stress level, or co-infections) (Robinson*et al*,2017). The pathogenicity of H. pylori and the range of virulence factors these bacteria generate receive the greatest attention among them. The presence of several adhesions that promote adhesion to the stomach mucosa, together with cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA), are the main factors that determine pathogenicity.

About half of the world's population is infected with Helicobacter pylori, a widespread bacterium that is more frequent in developing nations (Moayyedi and Hunt, 2004). Infection is more prevalent in underdeveloped nations (Pounder *et al*,1995), and the majority of people contract it during childhood (Malaty *et al*, 2002). According to simulations, H. pylori originated in East Africa and dispersed around 58,000 years ago. Since then, it has evolved into several strains with differing levels of virulence (Linz *et al*, 2007).

We still don't fully understand how *H. pylori* spreads. The human stomach is the only known reservoir. The oral-oral, fecal-oral, and gastric-oral routes are the ways that H. pylori is spread from person to person. The infection dosage for humans is minimal (Kusters *et al,* 2006), and it may be a sex-transmitted disease (Schütze *et al*, 1995, Singh *et al*, 1999).

By looking for IgG and IgA antibodies in patients' blood or serum, immune responses against *H. pylori* are used to identify infection. According to Malfertheiner *et al.* (2012), serology is the only test that is unaffected by the local alterations in the stomach that might result in a low bacterial load and false negative findings.

Antigens in stool samples are found using stool antigen assays (HpSA). When compared to polyclonal methods, ELISA formats including monoclonal antibodies against *H. pylori* proteins produced better findings (Paimela *et al*, 2006).

Serology and stool antigen tests differed significantly, according to *Naji et al*. (2014). In the serology test, 72 patients tested positive for H. pylori antibody (IgG) in their blood, accounting for 72% of the results, while 28% were negative. In the blood antibody test, the proportion of positive results was slightly higher in female patients (52, or 76.4%) than in male patients (22, or 68.7%). Of the 100 patients, 49 (49%) had a positive *H. pylori* infection diagnosis, whereas 51 (51%) had a negative stool antigen test result. The percentage of female patients who tested positive for the stool antigen was somewhat higher at 34 (50%) than the percentage of male patients, which was 14 (43.7%). 408 asymptomatic volunteers (aged 32.55 ± 11.98 years) participated in a significant study conducted in Nigeria by Ifeanyichukwu *et al.* 2018. The overall positivity rate of the H. pylori stool antigen test (28.2%) was significantly higher than the serology test (48.3%).

OBJECTIVES

The aims of the current study is to compare between stool antigen and blood antibody test method for detection of Helicobacter pylori which associated with several upper gastrointestinal disorders.

MATERIAL AND METHODS

Data collection

On the enrolment data, questionnaire forms were collected back and age and other data of each patient were noted. Blood was sampled from patients with one stool sample. Enzyme-linked immunosorbent assays (ELISA) were used to detect the levels of serum anti-*H. pylori* IgG and IgM antibodies. In addition, the stool samples were also assayed qualitatively using the monoclonal fecal 1H. pylori antigen rapid test kit.

Procedure

Stool samples were tested for H. pylori antigen using the monoclonal fecal H. pylori antigen rapid test kit (InTec PRODUCTS, INC.), following the manufacturer's instructions. Briefly, a small amount of stool sample was added to the test device, and the result was visually assessed after a specified time based on the appearance of a control line. Serum samples were tested for *H. pylori*-specific IgG and IgM antibodies using an enzyme-linked immuno-sorbent assay (ELISA). In the ELISA test, microplates were coated with H. pylori antigen, and diluted serum samples were added to the wells. If present, *H. pylori*-specific IgG and IgM antibodies bound to the antigen. After washing, an enzyme-conjugated secondary antibody was added, and the reaction was visualized using a chromogenic sub-strate. Absorbance was measured at a specific wavelength, and results were compared to calibrators and controls for interpretation**.**

RESULTS

The study group comprised 50 patients with a mean age of 38.82 years (10-70 years). The most common symptom encountered was epigastric pain, which was seen in all cases. All of these were tested by using two techniques; serological using ELISA technique for specific IgG and IgM antibodies against H. pylori. In addition, fresh stool samples were collected on the same day and tested for the qualitative detection of *H. Pylori* antigen which named *H. pylori* stool antigen (HpSA).

The result revealed that 23 (46%) of subject were positive for *H. pylori* infection by stool antigen test. In contrast, the serology test shows that 32 (64%) of cases was positive using IgG and 24 (48%) positive by using IgM test. The data presented in table1 and figures (1-3).

Table 1: Comparison between different dignosis method of H. Pylori

HpSA				IgG				IgM			
Positive		**Negative**		**Positive**		**Negative**		**positive**		**negative**	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
23	46%	27	54%	32	64%	18	36%	24	48%	26	52%

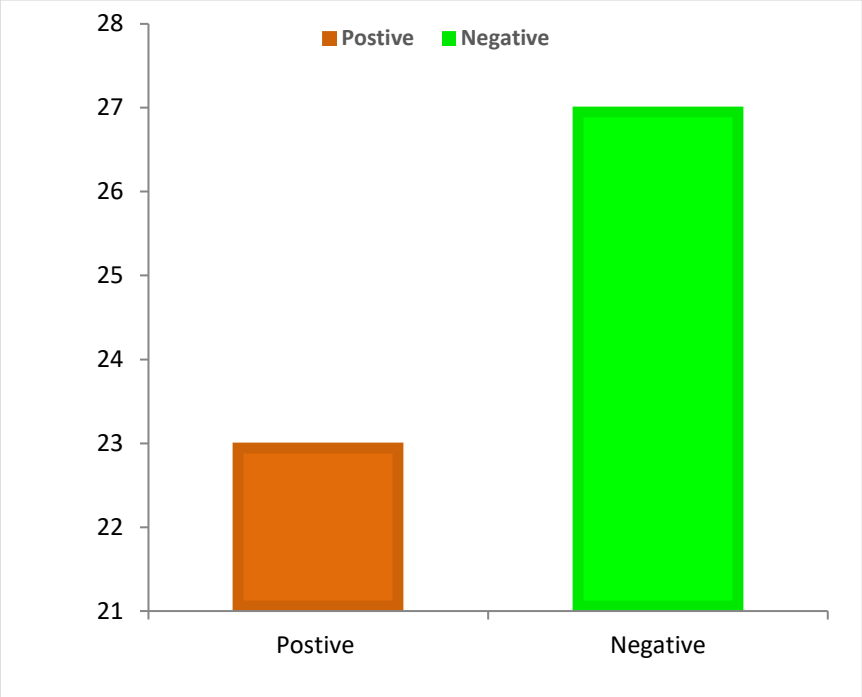

Figure 1: The percentage of postive and negative *H.pylori* infection diagnostic by HpSA Test.

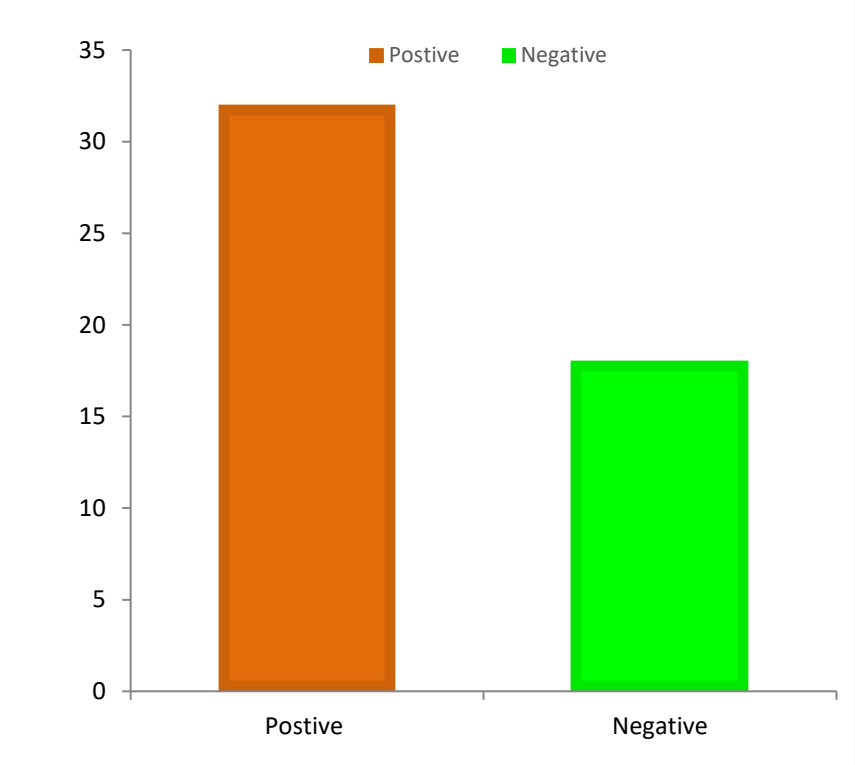

Figure 2: The percentage of postive and negative H.pylori infection diagnostic by IgG antibody.

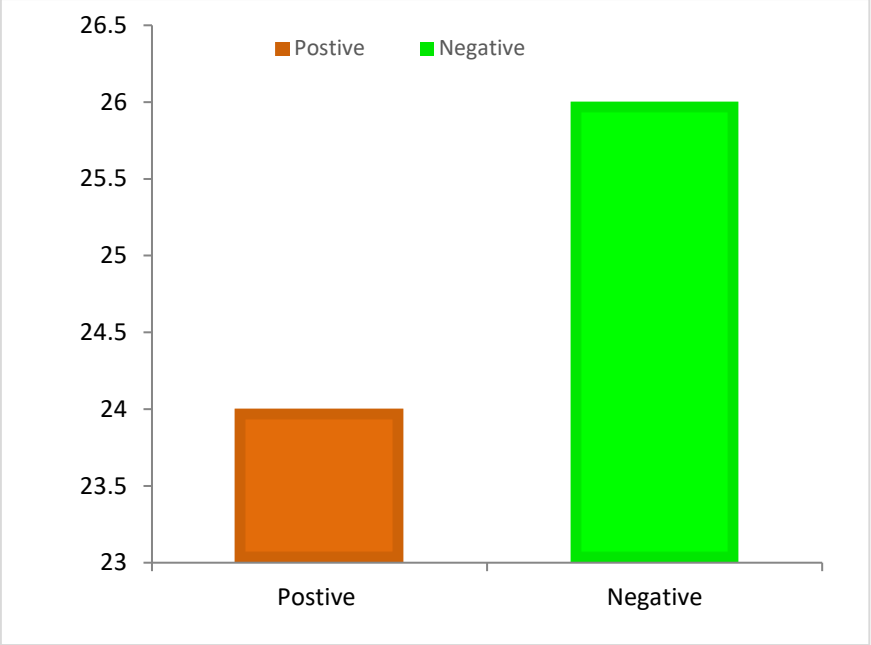

Figure 3: The percentage of postive and negative H.pylori infection diagnostic by IgMantibody

The stool antigen test showed that 10 cases (45.5%) out of 22 male patients were positive, whereas, in female 13 (46.4%) out of 28 cases were positive. In terms of serology test 15 (68.1%) cases out of 22 and 11 (50%) out of 22 male patients were positive using IgG and IgM respectively. in addition the serology test showed that in female cases were 17 (60.7%) cases out of 28 are positive by using IgG and 13(46.5%) out of 28 cases are positive using IgM. The data is shown in table 2 and figure (4-6).

Table 2. The prevalence of H.pylori infection in male and female.

Gender	HpSA			IgG			IgM		
	Positive	**Negative**	**Total**	**Positive**	**Negative**	**Total**	**Positive**	**Negative**	**Total**
Male	10 (45.5%)	12 (54.5%)	22	15 (68.1%)	7 (31.9%)	22	11 (50%)	11 (50%)	22
Female	13 (46.4%)	15 (53.6%)	28	17 (60.7%)	11 (39.3%)	28	13 (46.5%)	15 (53.5%)	28
Total	23	27	50	32	18	50	24	26	50

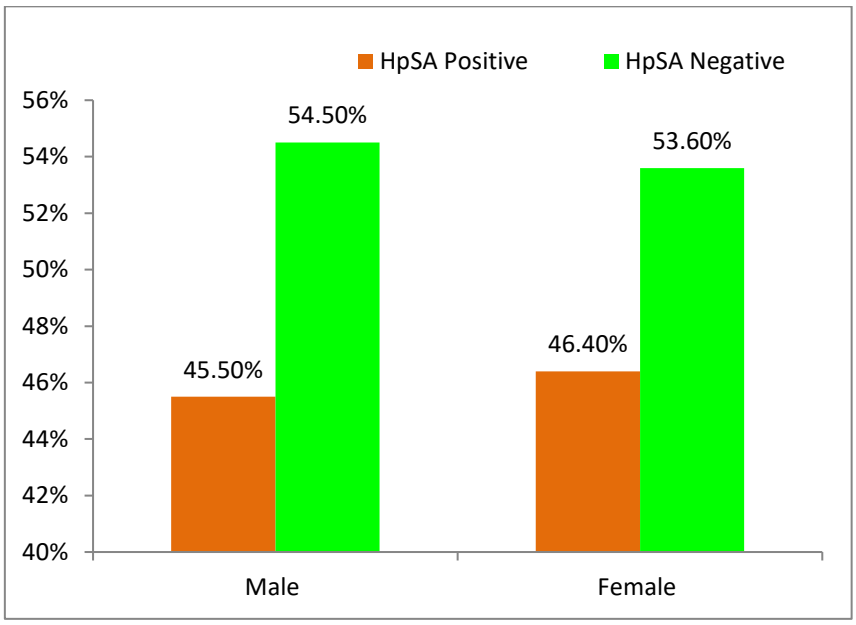

Figure 4: Sex distribution among patients analysed by HpSA Test.

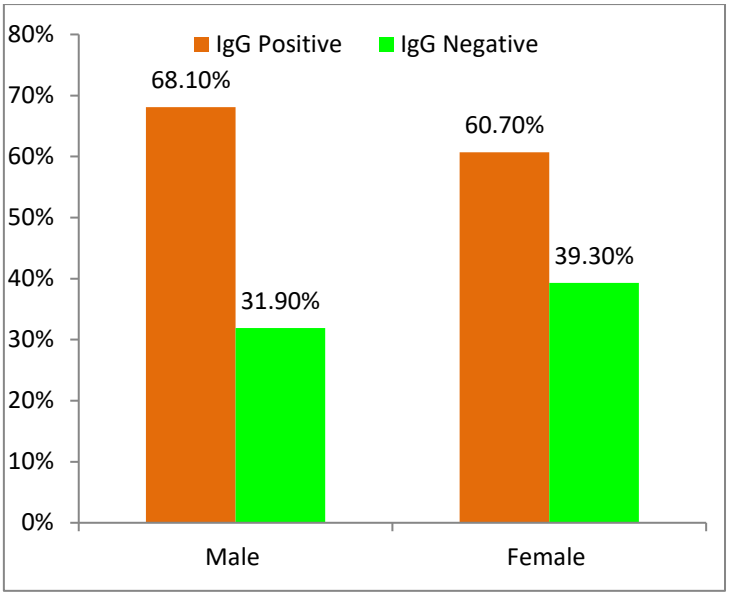

Figure 5: Sex distribution among patients analysed by IgG serology Test.

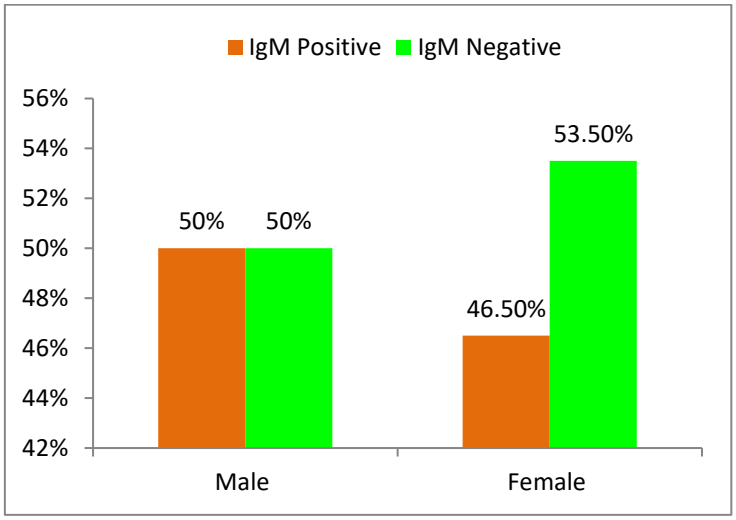

Figure 6: Sex distribution among patients analysed by IGM serology Test

In table 3 and figures (7-9) the serology (IgG) test indicated greater H. pylori positively compares with the stool antigen test in 21-30 years age group (14% vs. 12%); in 31-40 years age group (18% vs. 14%) and 51-60 years age group (8% vs. 6%). Data further indicated that the highest positive results was found in the age group of 31-40 years, which indicated 14% positivity by stool antigen test and 18% by the serology test.

Table 3: The prevalence rate of H.pylori in different age groups.

Age Groups	HpAS		IgG		IgM	
	positive	Negative	Positive	negative	Positive	Negative
10-20	0 (0%)	6 (12%)	3 (6%)	2 (4%)	3 (6%)	3 (6%)
21-30	6 (12%)	6 (12%)	7 (14%)	6 (12%)	7 (14%)	6 (12%)
31-40	7 (14%)	5 (10%)	9 (18%)	3 (6%)	5 (10%)	7 (14%)
41-50	5 (10%)	3 (6%)	5 (10%)	4 (8%)	5 (10%)	3 (6%)
51-60	3 (6%)	4 (8%)	5 (10%)	2 (4%)	2 (4%)	5 (10%)
>60	2 (4%)	3 (6%)	3 (6%)	1 2%)	2 (4%)	2 4%)

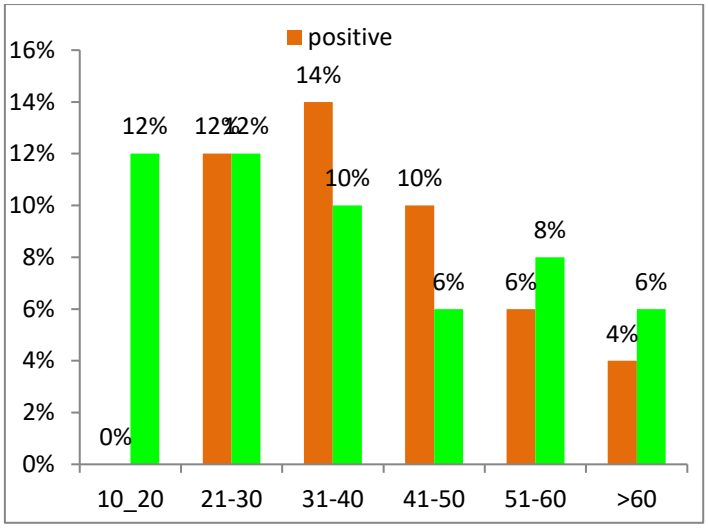

Figure 7: Age distribution among patients by HpSA Test.

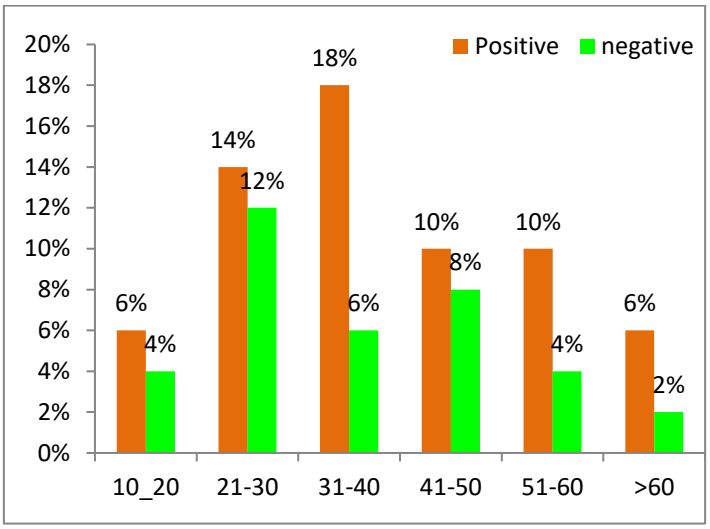

Figure 8: Age distribution among patints by IgG serology Test.

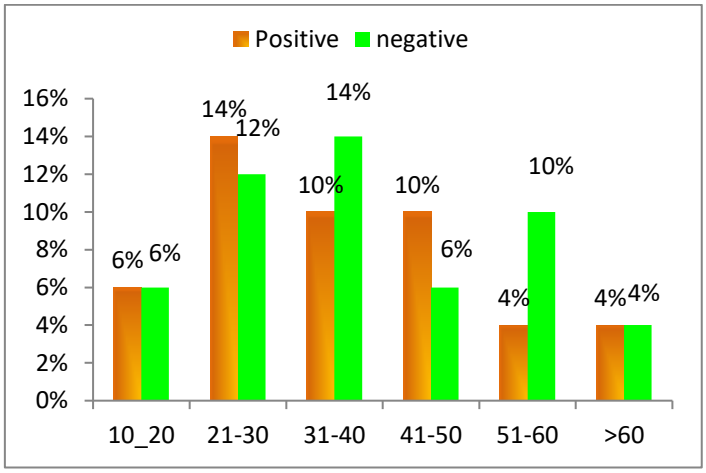

Figure 9: Age distribution among patients by IGM selology Test.

DISCUSSION

Since *Helicobacter pylori* infections are extremely prevalent in underdeveloped nations, a quick and inexpensive diagnostic method could be helpful in treating *H. pylori* infections in both adults and children from these areas. Libya is among the emerging nations where the frequency of *H. pylori* infection is rising along with the incidence of gastritis and duodenal ulcers. According to a study conducted in Libya, 82% of people had *H. pylori* (Bakka, and Salih, 2002). Hence the need

to re-appraise the performances of different cost-effective, easy to use methods in diagnosis of *H. pylori* infection in the general population.

The present study was under taken to compare the different diagnosticc methods, with non-invasive techniques (blood antibody and stool antigen test.methods) for detection of H. pylori infection in patients with gastritis. The current study included 50 patients with upper GIT symptoms proven to be with gastric affection in the form of Acid peptic disease.

As expected, the present study showed significant difference between serology (IgG) and stool antigen test. 23 patients out of 50 were H. Pylori positive by using stool antigen test with percentage 46%. In contrast, the serology test showed different result. 32 and 24 out of 50 patient were H. pylori positive by using IgG and IgM respectively. With percentage 64% for IgG and 48% for IgM.

In fact the result reflect the truth that serology test IgG may give a positive result in case of previous but not current infection. Furthermore, the level of *H. pylori* infection between IgM and stool antigen test shows just slight preponderance 48% and 46% respectively.

According to the 2014 study by Naji *et al*., the prevalence of *H. pylori* infection was 72% by serology test and 49% by stool antigen test, which is comparable to our 48% result. Additional research supports the earlier findings from Japan, where 61.4% and 56.4 of 994 patients, respectively, were found to be infected by stool antigen testing and serology. Sixty-four percent of the patients in the current study had a positive H. pylori infection as determined by a blood antibody test. These findings were comparable to those of Luthra *et al,* (1998), who reported a 63% positive outcome, while Satti *et al.* (2004) reported an 87.7% positive result. This discrepancy results from outdated research and ignorance about hygiene.

Additionally, we discovered that 46% of patients had a positive stool antigen test result for *H. Pylori* infection, which is less than the number reported by Chisholm *et al.* (2004). This was because the stool in the aforementioned study included more antigens, and their diagnostic techniques were more advanced than ours. There were fewer positive instances in our study, which might be because there was not enough antigen in the stools. Stool antigen test results from other studies show varying levels of Pylori infection, which may be caused by variations in kit quality and climate.

CONCLUSION

Blood antibodies test method showed greater number of positive cases (64%) than the stool antigen tests method (46%) which may be due to past infection. Stool antigen test, which detects present but not previous infection of *H. pylori*, would be more accurate than serology test.

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